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EFFECT OF DECOMPOSING ORGANIC MATTER ON THE SOLUBILITY OF CERTAIN INORGANIC CONSTITUENTS OF THE SOIL

By CHARLES A JENSEN,

Assistant in Plant Malnutrition Office of Biophysical Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In an investigation already reported [1] relative to the cause of mottle-leaf of Citrus trees in southern California, it was found that the percentage of mottling was inversely correlated with the humus content of the soil as measured by the amount of organic material extracted from the soil with a 4 per cent ammonium-hydrate solution, the calcium having previously been removed with hydrochloric acid. The examination of 120 orange groves in the Riverside, Cal., district showed a correlation of 0.67 between the mottling of the orange leaves and the reciprocal of the humus content in the soil. This association is further supported by the marked growth response of orange trees, especially on the clay-loam type in the district mentioned when irrigation water is supplied through basins mulched with organic material, the soluble organic products of the decomposition in the mulch being carried directly to the root system by the irrigation water.[2]

The marked growth response of Citrus trees following the addition of certain kinds of organic matter or of organic solutions derived from the decomposition of organic matter may be due to the addition of some organic or inorganic constituent, or possibly to some indirect action of the organic solution on the soil or the soil flora. The fact that chlorosis is sometimes associated with a deficiency of soluble iron, and that magnesium, according to the investigations of Willstätter [3] and his colleagues, is an essential constituent of chlorophyll, has made it appear desirable to determine if possible to what extent the addition of organic matter to the soil increases the solubility of these elements and other essential

[1] Briggs, L. J., Jensen, C. A, McLane, J. W. Mottle-leaf of Citrus trees in relation to soil conditions. *In* Jour. Agr. Research, v. 6, no. 19, p. 721–729. 1916.

[2] Briggs, L. J., Jensen, C. A, McLane, J. W. The mulched-basin system of irrigated Citrus culture and its bearing on the control of mottle-leaf. U. S. Dept. Agr. Bul. 499, 31 p., pl. 1. 1916.

[3] Willstätter, R. The chemistry of chlorophyll. *In* Rpt. 79th Meeting Brit. Assoc. Adv. Sci., 1909, p. 667–668. 1910.

inorganic plant constitutents. An economic bearing is also given to the investigation by the fact that the mulched-basin system has in some cases had a marked effect in reducing mottle-leaf on Citrus trees.

The present paper deals with the solvent action on certain inorganic soil constituents of the water-soluble decomposition products of manures and other organic fertilizers. Soils were extracted (*a*) with soluble organic matter obtained from decomposing green manures and from stable manures; (*b*) with soluble organic matter obtained from thoroughly decomposed green manures; (*c*) with artificially prepared humus solutions obtained by hydrolyzing organic substances with acids; and (*d*) with osmosed organic solutions derived from the decomposition of organic matter. In addition, stable and green manures were added directly to the soil and the effect of this treatment was noted (*a*) on the amounts of certain soil minerals dissolved out with water, and (*b*) on the change produced in the specific electrical conductivity of the soil.

METHOD USED IN DECOMPOSING ORGANIC SUBSTANCES

Green barley hay, sweet clover, and alfalfa were dried and chopped into small pieces, and 70 gm. of each of these substances were placed in separate large bottles, moistened to saturation with distilled water, and allowed to ferment. An equal amount of dry cow manure was similarly treated. Fourteen days later these four substances were shaken up thoroughly with 1,500 c. c. of distilled water each and the coarser material filtered out through muslin. This filtrate was then passed through a Chamberland porous filter and collected. The solid organic matter from each of the four substances was returned to its respective bottle, kept saturated, and at a later period was again shaken with distilled water as above, filtered, and the filtrate again collected. The organic solutions thus obtained were used for soil extraction.

TABLE I.—*Intervals between successive extractions of green manures*

No. of extract.	Date extracted.	Number of days since placing in bottles.	Number of days since preceding extraction.
1......	Feb. 2	14	....
2......	Feb. 24	36	22
3......	Mar. 15	55	19
4......	Apr. 25	96	41

These organic extracts were entirely free from suspended matter. The intervals between extractions are given in Table I. The organic solutions, or solvents,[1] were added to the soils under investigation in the proportion of 250 gm. of soil to 500 c. c. of solvent.

[1] In order to avoid confusion in the mind of the reader, the term "solvent" will be used in speaking of the organic extracts obtained from the various organic substances, and which are used in making soil extracts. Relative to the soil, they are solvents, though in themselves they are systems of water-solvent and organic and inorganic solutes.

METHOD OF ANALYSIS

The soil extracts thus obtained contained large amounts of organic matter in solution, which it was necessary to remove before proceeding with the analysis. A number of methods were tested with solutions containing known amounts of the mineral elements under investigation, together with large amounts of organic extracts known to be free from these mineral elements. Of these methods, the following gave the best results:

The soil extracts obtained with the organic solvents were made and kept slightly ammoniacal, an excess of ammonium oxalate was added, and the extracts were evaporated to dryness. The residue was ignited just sufficiently to burn off the organic matter. The ignited mass was taken up with a measured [1] amount of nitro-hydrochloric acid, diluted somewhat with distilled water, and heated on the water bath until everything except the free silica had gone into solution. This acid solution was made up to volume and analyzed.

The phosphoric acid was determined volumetrically by Pemberton's molybdic method. The calcium was determined volumetrically by titrating the oxalate with potassium permanganate. The magnesium was determined volumetrically by the method of Meade. The detailed methods of determining the above-mentioned three elements are substantially those given by Sutton.[2] The iron was determined colorimetrically by comparing the red color developed on the addition of potassium thiocyanate to the unknown solution with a standard iron solution. The color in the standard solution was developed at the same time that the color in the unknown solutions was developed, and the readings were made at once.

SOLUBILITY OF SOIL MINERALS IN EXTRACTS OF DECOMPOSING ORGANIC MATERIAL IN DIFFERENT STAGES OF DECOMPOSITION

In the mulched basin under field conditions the products of decomposition are gradually leached into the soil. To approximate this action, the organic solvents were prepared by extracting the same samples of decomposing organic matter at intervals as shown in Table I. The amount of calcium, magnesium, phosphoric acid, and iron removed from two soils by such organic solutions prepared from cow manure, barley hay, alfalfa hay, and sweet-clover hay in various stages of decomposition is given in Table III.

To determine the solvent action of these organic solvents in excess of that of pure water alone, a number of determinations were made of the solubility of the iron, calcium, magnesium, and phosphoric acid in the

[1] It was necessary to use a measured amount of acid in taking up the ignited residue after evaporating the organic soil extracts to dryness, owing to the fact that the red solution formed by the addition of potassium thiocyanate to ferric iron is rendered colorless by an excess of hydrochloric or nitric acid. In working with a few parts per million of iron in the solution, it was found unsafe to have present in 100 c. c. of the solution under investigation more than 3 c. c. of concentrated nitric or hydrochloric acid, or the same amount of a mixture of both acids.

[2] Sutton, Francis. A Systematic Handbook of Volumetric Analysis . . . ed. 10, 621 p., 121 fig. 1911.

soils under examination, using distilled water only as a solvent. The means of these determinations are given in Table II.

TABLE II.—*Average amounts of minerals removed by distilled water from the soils used*

[Results expressed as parts per million of dry soil]

	Clay loam.				Sandy loam.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Average amounts removed	0.47	43	12	24	0.36	39	7	14
Number of determinations in average	13	13	11	11	13	10	10	10

These mean values have been deducted in each instance from the solubility of the corresponding element in the presence of the organic solvent.

The organic solvents themselves all contained calcium, magnesium, iron, and phosphoric acid in solution, so that it was necessary to determine the amount of each of these elements added to the soil along with the organic matter. These determinations are given in the first part of Table III, all results being expressed in parts per million of the weight of the dry soil used. The second part of Table III shows the concentration of each element in the organic solution after it had been shaken with the soil and freed from suspended material by filtration through a Chamberland tube. In each instance, however, the amount of each element dissolved in distilled water, as given in Table II, has been deducted.

TABLE III.—*Minerals removed from soils by extracts of organic substances during various stages of decomposition*

[Results expressed in parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substances and their successive extraction.	Inorganic substances added to soil with organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Barley:												
First	7.00	13	76	138	0.21	508	105	51	1.69	529	66	93
Second	6.00	106	38	69	4.90	358	89	105	3.96	368	49	81
Third	2.40	58	20	26	.84	232	57	14	2.04	225	41	21
Fourth	.75	55	6	5	.08	78	5	−2	.09	94	5	11
Sweet clover:												
First	14.00	59	122	280	3.34	402	129	184	4.80	261	103	195
Second	23.30	125	40	60	1.00	473	132	53	1.46	512	92	77
Third	8.40	149	20	24	−.02	185	52	2	.19	217	32	11
Fourth	1.10	86	5	10	−.25	91	12	9	.89	99	7	13
Alfalfa:												
First	2.25	211	150	184	.66	472	157	88	.68	426	75	159
Second	.75	264	59	66	.50	523	131	71	.96	480	70	72
Third	.50	79	16	23	.03	195	58	22	.61	166	21	21
Fourth	.30	95	10	7	−.42	133	13	13	−.11	122	3	14
Cow manure:												
First	.84	91	82	135	1.46	292	92	49	1.41	211	135	103
Second	1.17	159	78	153	.45	216	73	96	.13	185	51	125
Third	.91	74	36	115	.03	84	30	43	.27	99	21	71
Fourth	.26	114	8	67	−.08	83	10	25	.05	105	0	43

The various organic solvents are seen in Table III to exert a marked solvent action on the calcium and magnesium in soils. This action diminishes in intensity with the successive extracts, especially after the second. The mineral content of the organic solvents also decreases in the successive extracts, and these inorganic components doubtless play some part in the solubility of the soil minerals which can not be differentiated at this stage from the solvent action of the organic compounds.

The total amount of the inorganic elements dissolved from each soil by the four extractions, as well as the total amount of the same elements added to the soils with the organic solvents, is given in Table IV. It will be seen that the total amount of phosphoric acid and of iron recovered from the soil extracts did not equal the amount present in the organic solvents, and so added to the soil in making the extract of the latter. But the amount of these two elements dissolved from the soil was greatly in excess of the amount made soluble by water alone. In all cases the amount of calcium dissolved from the soil was much more than that added in the organic solvent, and in most cases also more magnesium was recovered than was added in the organic solvent.

TABLE IV.—*Total amounts of minerals removed from soils by the four successive organic extracts of freshly decomposing organic matter*

[Results expressed in parts per million of dry soils. Amounts removed by distilled water have been deducted]

Organic substance.	Inorganic substances added to soil with the organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Barley	16.15	232	140	238	6.03	1,176	256	168	7.77	1,206	161	206
Sweet clover	46.80	419	187	374	4.07	1,151	325	248	7.44	1,089	234	297
Alfalfa	3.80	649	230	280	.77	1,323	202	194	2.14	1,194	169	366
Cow manure	3.18	438	204	470	1.86	675	205	213	1.86	600	207	343

It is evident from Table IV that the organic solvents obtained from the decomposing green manure were more effective in removing calcium than the organic solution obtained from the cow manure. Walters [1] has shown that acetic acid and proprionic acid are formed in considerable quantities through the decomposition of green rye or green alfalfa, and the greater solvent action of the solutions of green manures is perhaps attributable in part to the presence of such acids. The amount of calcium or magnesium added to the soil with the organic solvents bears no relation to the amount of these elements recovered in the soil extracts. The amount of phosphoric acid recovered varies directly with the amount added to the soil in the organic solvent. The various organic solvents

[1] Walters, E. H. The presence and origin of volatile fatty acids in soils. (Abstract.) *In* Science, n. s. v. 44, no. 1128, p. 217. 1916.

obtained from decomposing green manures, whether derived from a non-leguminous or a leguminous crop, showed but slight differences in their solvent action upon the elements under examination.

The total quantity of soil treated with the organic solvents would, in the case of the four materials studied, correspond approximately to a ratio of 2 parts of dry organic material to 100 parts of dry soil. The organic material was by no means completely decomposed at the time the last extraction was made. The amount of these elements dissolved from the soil as shown in Table IV was obtained during the 96 days the decomposition was in progress. We may therefore look upon the results in Table IV as representing roughly the gain in the soluble iron, calcium, magnesium, and phosphoric acid resulting from the addition of 2 per cent of organic matter to the soil, the amount dissolved from the soil by distillled water alone having been deducted. These figures show that the addition of organic matter to the soil markedly increased the amount of soluble iron, calcium, magnesium, and phosphoric acid. Expressed in pounds per acre-foot, the amounts range as follows:

Iron	pounds..	3 to 31
Calcium	do....	2,400 to 5,300
Magnesium	do....	640 to 1,300
Phosphoric acid	do....	670 to 1,400

These figures do not necessarily represent an increased solubility of the soil components, however, except in the case of calcium and magnesium. The amount of iron or phosphoric acid recovered from the soil was in no instance equal to the amount added to the soil in the organic solvent.

Separate solubility experiments were made by adding to the untreated soils the same amount of the elements under investigation as were added to the soils with the organic solvents. The salts, used individually, were the sulphates of iron, calcium, and magnesium, and dibasic sodium phosphate. Practically all the iron and about two-thirds of the magnesium and phosphoric acid added remained in the soil. On the other hand, the addition of calcium sulphate increased the solubility of calcium and magnesium, and the addition of magnesium sulphate increased the solubility of calcium.

From the results obtained it was impossible to differentiate between the solvent action of the organic and inorganic compounds in the organic solvents.

REACTION OF ORGANIC EXTRACTS TO INDICATORS

The organic solvents obtained from the decomposing green manures used in making soil extracts were examined as to their reaction. None of the organic solvents obtained from the first extraction after 14 days' decomposition showed an alkaline reaction with phenolphthalein or an acid reaction with methyl orange, and only occasionally was a red color

developed by boiling the extract in the presence of phenolphthalein. However, it required from 0.8 to 2.8 c. c. of normal hydrochloric acid per 100 c. c. of extract to develop an acid reaction in the extracts, using methyl orange as an indicator. Table V shows the results of the titrations of the first set of organic solvents both before and after passing through the soils.

TABLE V.—*Titration of solutions of organic substances before and after extracting soils*

Decomposing substance from which organic solution was obtained.	Normal hydrochloric acid required to develop an acid reaction in 100 c. c. of solution, using methyl orange as an indicator.		
	Organic solution.	After extracting.	
		Clay loam.	Sandy loam.
	C. c.	*C. c.*	*C. c.*
Barley hay	0. 80	1. 00	1. 00
Sweet-clover hay	2. 80	2. 60	2. 40
Alfalfa hay	2. 40	1. 90	2. 60
Cow manure	1. 70	1. 70	1. 70

There were substances, probably of organic nature, present in both the organic solvents and the soil extracts obtained with these solvents, that were neither alkaline to phenolphthalein nor acid to methyl orange. These substances, however, were capable of combining with a considerable amount of hydrochloric acid before an acid reaction was shown, using methyl orange as indicator. The amount of acid thus required varied approximately with the amount of calcium present in the solvents and extracts. This latter fact, however, does not account entirely for the comparatively large amount of acid required to make these solutions acid. Solutions from hydrolyzed organic substances containing none of the elements under investigation required about the same amount of acid to develop an acid reaction, using methyl orange as an indicator.

ARTIFICIALLY PREPARED MANURES

During the summer of 1915 various green-manure substances, including barley hay, sweet clover, bean straw, and alfalfa, were dried, placed in 4-gallon glazed jars, saturated with water, and allowed to ferment. At the same time a jar was filled with dry cow manure and similarly treated. Dried hay, leaves, weeds, etc., soon break down under these conditions, and in a surprisingly short time decompose into a mass closely resembling manure. Six months after these substances had been placed in the jars decomposition had evidently gone to an end, except for the slow changes which ordinarily follow the first rapid bacterial action in any manure kept in tanks.

Organic solvents obtained from these thoroughly decomposed substances were made by shaking up an equivalent of 33 gm. of dry material in 500 c. c. of distilled water, and filtering through Chamberland filters. These organic solvents were then used for making soil extracts in the proportion of 500 c. c. of the solvent to 250 gm. of soil. These soil extracts were filtered through Chamberland filters and analyzed (Table VI). The results given in Table VI show that in most instances the organic solvents obtained from the completely decomposed green manures were not as active in breaking up the soil minerals as the organic solvents from the same materials in the first stages of decomposition (see Table III).

TABLE VI.—*Soil minerals removed by extracting soils with organic solvents from completely decomposed artificial manures*

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substance.	Added to soil with organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Barley hay	0.25	104	94	81	0.65	305	92	43	1.69	205	68	91
Sweet-clover hay	3.70	116	140	105	1.89	472	196	77	3.32	292	140	79
Alfalfa hay	3.20	71	83	110	1.62	411	149	30	1.41	246	93	77
Cow manure	.18	89	120	93	.18	290	116	83	.09	221	97	64
Bean straw	.98	834	247	82	.05	758	385	31	1.83	670	315	55

The organic extracts of the completely decomposed green manures were neither acid to methyl orange nor alkaline to phenolphthalein, but required a considerable amount of acid to develop an acid reaction, with methyl orange as an indicator (Table VII).

TABLE VII.—*Quantity (in cubic centimeters) of normal hydrochloric acid required to develop an acid reaction in 100 c. c. of extract from completely decomposed green manures before and after extracting soils, using methyl orange as an indicator*

Source of extract.	Organic extract.	After extracting.	
		Clay-loam soil.	Sandy-loam soil.
	C. c.	*C. c.*	*C. c.*
Barley manure	2.0	([a])	1.6
Sweet-clover manure	([a])	Trace.	Trace.
Alfalfa manure	3.8	3.2	3.3
Cow manure	1.6	1.6	1.5
Bean-straw manure	5.8	4.8	5.1

[a] Not determined.

ORGANIC LIQUID FROM COMPOSTED ALFALFA

In the summer of 1915 a bale of alfalfa was placed in a galvanized tank fitted with a cover, moistened, and allowed to decompose. Water was occasionally added, and a quantity of liquid accumulated in the tank which possessed the very dark color and ammoniacal odor typical of a barnyard compost heap from which the liquid material does not escape.

This liquid was used, after some dilution, for making soil extracts. To a second portion of the liquid about 50 per cent, by volume, of alcohol was added, which precipitated part of the organic matter. This was filtered, and the filtrate and precipitate were used separately for making soil extracts, the alcohol first being driven off. The precipitate was fairly soluble in water.

A third portion of the original liquid was dialyzed through rather close-textured parchment tubing, the dialysate being frequently removed and replaced with distilled water. Both the dialysate and the organic residue were employed in making soil extracts. The organic residue in this case formed a colloidal suspension rather than a true solution in distilled water. The results of the analyses of the soil extracts made with these organic separations are presented in Table VIII.

The concentration of these various organic solvents with respect to the original alfalfa liquid was the same in all cases.

TABLE VIII.—*Soil minerals removed by extracting soils with the liquid resulting from decomposing alfalfa*

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substance.	Inorganic substances added to soil with organic solvents				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Untreated liquid[a]	1.78	30	30	57	0.72	389	103	18	0.26	209	43	18
Alcohol soluble[a]	3.45	30	20	50	.28	645	167	10	.62	260	43	18
Alcohol insoluble[a]	2.20	32	15	65	.46	164	54	4	.25	96	19	6
Dialysate	.65	30	23	26	.98	510	136	4	1.78	294	47	10
Dialyzed residue	4.36	19	12	54	.88	25	11	15	1.45	6	−3	10

[a] Average of two separate determinations with two separate samples of the solvents.

All these solvents, excepting the dialyzed residue, removed considerable amounts of calcium from the soils. The magnesium was quite freely removed from the heavy soil, but less so from the light soil. Phosphoric acid was not recovered in amount equal to that added to the soil with the organic solvents, but an increase over the amount removed by distilled water was obtained. The alcohol-insoluble organic material was not as

effective in dissolving the minerals as the alcohol-soluble portion, and the organic residue from the dialyzer was the least effective. As stated above, this material when taken up with distilled water was chiefly in a state of colloidal suspension, and a strong solvent action could hardly be expected.

The amount of inorganic elements added to the soils with these different organic solvents was about the same in all cases. It would therefore appear that the solvent action of these different organic solvents was due largely to the effect of the different organic constituents present in the solvents.

The untreated organic liquid was strongly ammoniacal, requiring 4.2 c. c. of normal hydrochloric acid per 100 c. c. of solution to dispel the red color of phenolphthalein at room temperature.

HYDROLYZED ORGANIC MATTER

It is recognized that in the preceding experiments the inorganic salts present in the organic solvents may have exerted a solvent action on the soil minerals. In order to eliminate this effect, soluble organic extracts were prepared free from the inorganic elements under investigation.

Dried alfalfa, sweet clover, barley hay, and sugar were digested for about 24 hours on the water bath with hydrochloric acid of about 1.115 specific gravity. The acid was then washed out, the residue extracted with 4 per cent ammonia, and the solid insoluble material filtered out. The ammonia extract was then heated on the water bath, the water lost by evaporation being occasionally partly replaced, until all free ammonia was driven off. The solutions were found by analysis to be free from iron, calcium, magnesium, and phosphoric acid. They were then standardized gravimetrically. In addition to the above solutions, an organic solution was also prepared from horse manure. It was washed thoroughly with hydrochloric acid until no more calcium came through the filter. The acid was then washed out and the residue extracted with ammonia. The ammonia was driven off and the resulting organic solution standardized and used in soil extraction.

It will be seen from Table IX that the mineral-free organic solvents here used were quite effective as solvents. The sugar humus was most effective in dissolving magnesium and calcium. It also increased the solubility of the iron slightly. Its effect on the solubility of the phosphoric acid in the soils was negligible. Some of the other solvents, however, exerted a marked solvent action on the phosphoric acid in the soil.

The results obtained with the mineral-free humus solutions show clearly a solvent action on the iron, calcium, magnesium, and phosphoric acid in the soil.

TABLE IX.—*Amounts of soil minerals removed from soils by solutions of artificial humus or hydrolyzed organic substances*

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Source of humus.	Organic matter in solvent.	Clay-loam soil.				Sandy-loam soil.			
		Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
	Per ct.								
Barley hay	0.005	....	....	....	....	0.05	0	....	....
	.02	....	....	....	....	.19	20	....	....
	.04	....	....	....	....	.22	38	....	....
	.10	−0.08	108	33	21	11.64	98	2	22
Sweet-clover hay	.01	.06	8	9	−8	−.22	9	0	3
	.03	.06	25	20	2	.14	26	5	2
Alfalfa hay	.005	.02	12	....	....	−.09	2	....	....
	.02	.21	35	....	....	−.08	12	....	....
	.04	.28	65	....	....	−.11	22	....	....
	.01	.25	−4	....	....	.16	1	2	12
	.03	.87	34	....	....	.09	18	6	8
	.10	−.08	94	33	5	.39	66	5	9
Sugar	.01	.20	56	16	0	.22	160	....	....
	.03	.29	150	49	−2	.80	242	....	....
Horse manure	.005	....	....	....	....	.09	10	....	....
	.02	....	....	....	....	.23	45	....	....
	.04	....	....	....	....	.48	53	....	....
	.10	1.72	115	19	53	8.64	48	3	81

Like the organic extracts of the freshly decomposing green manures, these hydrolyzed humus solutions were neutral to phenolphthalein and methyl orange. They required, however, about the same amount of hydrochloric acid as did the above-mentioned organic extracts, in order to show an acid reaction with methyl orange.

SOLVENT ACTION IN SOILS OF DECOMPOSING GREEN MANURES

In the study of the humification of organic substances in soils in pots, several kinds of green-manure substances were used, and to some of these pots certain inorganic salts were also added. These soils were kept moist and stirred occasionally. Samples were extracted with distilled water and the extracts analyzed for the mineral elements discussed in the preceding work. Table X shows the results obtained in one of these experiments after the organic matter had been in contact with the soil for six months. The alfalfa was slightly more effective in bringing the soil minerals into solution than the manure.

Table XI shows the results from a similar experiment with lighter soils, in which the organic matter had been in contact with the soil for three months. In this case the sweet clover did not exert as pronounced a solvent action as the manure. An increasing solvent action is shown with an increasing amount of organic matter.

TABLE X.—*Solubility of soil minerals as effected by the addition of organic manures, lime, gypsum, and sodium carbonate six months after the addition of substances to the soil*

[Results expressed as parts per million of dry soil]

Soil treatment.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Control (nothing added)	0.75	25	7	16
3 per cent horse manure only	.39	42	7	38
3 per cent horse manure plus 3 per cent calcium carbonate	.30	46	8	32
3 per cent horse manure plus 0.2 per cent sodium carbonate	.51	33	9	38
3 per cent horse manure plus 3 per cent calcium sulphate	Trace.	1,070	81	25
Control (nothing added)	.51	23	5	17
3 per cent alfalfa only	.39	53	12	25
3 per cent alfalfa plus 3 per cent calcium carbonate	.60	48	7	33
3 per cent alfalfa plus 0.2 per cent sodium carbonate	.69	39	7	30
3 per cent alfalfa plus 3 per cent calcium sulphate	3.00	925	63	18

TABLE XI.—*Solubility of iron and calcium in soils as effected by the addition of organic substances three months after the addition of substances to the soil*

[Results expressed as parts per million of dry soil]

Soil treatment.	Soil No. 1.		Soil No. 2.	
	Iron.	Calcium.	Iron.	Calcium.
Control (nothing added)	0.50	44	0.80	21
Cow manure, 0.2 per cent	.65	49	.80	15
Cow manure, 1 per cent	.90	53	.90	34
Cow manure, 3 per cent	2.50	58	1.57	41
Sweet-clover hay, 1 per cent	.65	29	.77	21
Sweet-clover hay, 3 per cent	.77	40	1.53	35

In another experiment two types of soil from two orange groves near Riverside, Cal., were treated with different kinds of organic matter in different amounts, with and without the addition of certain inorganic substances. These soils were put into nonsoluble containers, placed in a greenhouse in Washington, D. C., kept moist, and stirred occasionally for a period of about 6 months. The soils were then sampled and the specific electrical conductivities determined. The results are shown in Tables XII and XIII. The specific-conductivity figures have all been multiplied by 10^5.

The presence of organic matter considerably increased the amount of electrolytes in both soils. Three per cent of organic matter increased the conductivity more than did 1 per cent, and alfalfa produced a greater

solvent action than did the same quantities of barley or manure. Barley and manure in the same amounts had about equal effect in increasing the conductivity of the soils to which they were added.

TABLE XII.—*Effect of the addition of organic matter and inorganic minerals on the specific conductivity of sandy loam soil kept moist in the greenhouse for six months* [a]

Inorganic minerals.	Amount and kind of organic matter added.						
	None.	Manure.		Barley.		Alfalfa.	
		1 per cent.	3 per cent.	1 per cent.	3 per cent.	1 per cent.	3 per cent.
None	48	68	93	60	100	123	150
1 per cent of calcium carbonate	48	......	100	......	125	......	203
3 per cent of calcium carbonate	55	......	115	......	118	......	156
0.2 per cent of sodium nitrate	165	......	207	......	163	......	183
3 per cent of calcium sulphate	73	......	143	......	125	......	210

[a] Conductivity figures have been multiplied by 10^5.

TABLE XIII.—*Effect of the addition of organic matter and inorganic minerals on the specific conductivity of clay-loam soil kept moist in the greenhouse for six months* [a]

Inorganic minerals.	Amount and kind of organic matter added.						
	None.	Manure.		Barley.		Alfalfa.	
		1 per cent.	3 per cent.	1 per cent.	3 per cent.	1 per cent.	3 per cent.
None	83	88	143	115	155	165	250
1 per cent of calcium carbonate	75	......	155	......	135	......	230
3 per cent of calcium carbonate	88	......	113	......	138	......	238
0.2 per cent of sodium nitrate	185	......	223	......	215	......	325
3 per cent of calcium sulphate	120	......	135	......	175	......	288

[a] Conductivity figures have been multiplied by 10^5.

The addition of calcium carbonate to the heavy soil treated with organic matter caused a slight decrease in the amount of soluble salts liberated as compared with the effect of the organic matter alone. In the light soil treated with organic matter the addition of lime increased slightly the amount of soluble salts. On the whole, however, the presence of calcium carbonate did not greatly modify the formation of soluble salts in the soil.

When gypsum was added to the soil with the organic matter, the amount of soluble salts was increased beyond the increase produced when the same amount of calcium carbonate was added. This, however, is chiefly due to the greater solubility of gypsum. The solubility of gypsum is about 2 parts in 1,000, while that of calcium carbonate is much less. This is brought out also in Tables XII and XIII, which show that when 3

per cent of lime alone is added to the soil the conductivity is but slightly increased. The same amount of gypsum under the same conditions increases the conductivity about one-third.

The net effect of the organic matter in increasing the conductivity of the soil when calcium carbonate was added was about the same as when gypsum was added. This is seen by deducting the conductivities of the soils to which calcium carbonate alone was added from the conductivities of the soils to which both organic matter and calcium carbonate were added. Making similar subtraction of conductivities of the soils treated with gypsum, with and without organic matter, gives about equal conductivity results.

The addition of sodium nitrate to soils increased the conductivity from 2.5 to 3.5 times over the conductivities of soils receiving no treatments. However, when the conductivities of the soils receiving this salt alone are deducted from the conductivities of the soils receiving both organic matter and sodium nitrate it is seen that the solvent action of the organic substances in the presence of this salt is less than when the organic substances alone are present.

It is seen that the solvent action of 3 per cent of alfalfa mixed with the sandy-loam soil is almost as great as when the same soil is mixed with 0.2 per cent of sodium nitrate alone. The solvent action of 1 per cent of alfalfa mixed with the clay-loam soil was almost as great as when 0.2 per cent of sodium nitrate was added alone, and 3 per cent of alfalfa mixed with the clay-loam soil produced a solvent effect 1.4 times greater than when 0.2 per cent of sodium nitrate alone was added to the soil.

SUMMARY

In southern California many Citrus groves are now being operated under the mulched-basin system. The principal substances employed as mulching material in the basins are stable manure, alfalfa hay, barley hay, sweet clover, and bean straw. These substances soon begin to decompose when wetted by rains or irrigation water, and the decomposition products leach into the soil. On certain soil types this method has produced very marked improvements in tree growth and fruit setting, especially when the mulches used have been alfalfa or bean straw. The present paper is concerned with an attempt to determine the extent to which the beneficial action of the decomposing organic mulches may be ascribed to a solvent action on the soil minerals, resulting in the liberation of plant food.

Soil extracts were made with organic solvents obtained from freshly decomposing alfalfa hay, sweet clover, and barley hay. The same sample of organic matter was extracted four times at intervals of from three to six weeks, the sample being kept under conditions favorable to decomposition between the intervals of extraction. These organic solvents

were used immediately for soil extraction. Two types of soil, a clay loam and a sandy loam, were used.

In the four soil extractions these organic solvents removed from the soil from two to five times as much calcium as was added to the soil with the solvents. In most cases these solvents removed more magnesium from the soil in the four extractions than was added with the solvents, the increase varying from a small fraction to about 80 per cent. The amount of iron and phosphoric acid removed from the soil by these organic solvents in the four soil extractions did not equal the total amount added to the soil with the solvents. However, the amount of iron dissolved from the soil by the organic solvents exceeded the amount dissolved by distilled water from 1 to 5.5 times. The amount of phosphoric acid dissolved from the soil exceeded the amount dissolved by water from 1.7 to 5.4 times. These various organic solvents, whether derived from a leguminous or a nonleguminous crop, had about equal solvent action on the soil minerals.

Organic solvents obtained from cow manure treated in a manner similar to the freshly decomposing green manures did not dissolve as much calcium from the soil as the solvents derived from the latter substance. They exerted about the same solvent action, however, on the other elements under investigation.

The solvent action of these organic extracts on the soil minerals appeared to be due both to the inorganic salts present in the organic solvents and to the organic compounds.

Green manures kept moist until thoroughly decomposed gave organic solvents which removed calcium from the soil in amounts several times that added with the organic solvents. These solvents also removed magnesium, phosphoric acid, and iron considerably in excess of the amount dissolved by water alone.

The organic solvents showed no alkaline reaction with phenolphthalein nor acid reaction with methyl orange.

Three per cent of green manures and stable manure mixed with soil and allowed to undergo partial decomposition increased the solubility of calcium and phosphoric acid in the soils from 30 to 100 per cent.

Artificial humus solutions free from calcium, magnesium, iron, and phosphoric acid were prepared by hydrolyzing green manures and sugar with strong acid, washing them free from acid, and extracting with ammonia. These organic solvents, when freed from ammonia, increased the solubility of calcium in the soil, compared with its solubility in water, by amounts varying from a few parts to 240 parts per million of soil. They also increased the solubility of magnesium, phosphoric acid, and iron, but to a less extent.

In brief, the solubility of calcium, magnesium, iron, and phosphoric acid in Citrus soils of the Riverside district is measurably increased by the addition of green manure, stable manure, or their extracts. This

increase in solubility is due in part to the action of the inorganic salts contained in the organic substances or their extracts and in part to the solvent action of the soluble organic compounds formed during organic decomposition. The fact that a deficiency in soluble iron is known to induce certain types of chlorosis suggests that the beneficial effects following the addition of organic matter to Citrus soils may have been in part due to its solvent action on iron and other soil compounds. Such an effect, if it exists, has not yet been differentiated from beneficial effects resulting directly from the organic material added.

XYLARIA ROOTROT OF APPLE[1]

By FREDERICK A. WOLF, *Plant Pathologist*, and RICHARD O. CROMWELL, *Assistant Plant Pathologist, North Carolina Agricultural Experiment Station*

INTRODUCTION

During the year 1913, specimens of diseased apple roots (*Malus sylvestris*) were received for examination at the North Carolina Experiment Station. These roots were found to be invaded in a characteristic manner by a fungus, which was suspected of being the cause of death of the tree from which the specimens were taken. Identification of this organism was impossible, since no fruit bodies could be found; but it was realized that the fungus exhibited points of difference from those ordinarily recognized as the cause of apple rootrots. When, however, in the spring of 1914, other specimens of the same disease were received, the fungus was isolated in pure culture by the planted-plate method. Since this fungus was very evidently different in its cultural characters from familiar forms associated with the decay of apple roots and since no report of a similar disease could be found in the literature at hand, a study of the identity and pathogenicity of this organism was begun. A preliminary report, in abstract,[2] of this work has appeared under the title "Black rootrot of apple." Investigations of this disease have been in progress more or less continuously for about three years, and it is deemed advisable to present at this time the data on hand relative to the distribution and symptoms of the disease and the identity, cultural characters, and pathogenicity of the causal organism.

DISTRIBUTION OF THE DISEASE

It has been impossible thus far to make a careful survey in order to determine the exact distribution of this disease in North Carolina. Collections made by the junior author in 1914 show that the disease occurs in Haywood and Wilkes Counties. In addition, specimens were received during the same year from a correspondent in Polk County. During the following year the same disease was again collected in several localities in Wilkes County and also in the counties of Alexander, Surry, and Warren. In 1916, collections were made in Henderson County and from new localities in Wilkes and Haywood Counties. Since the places of collection are so widely separated and since no particular difficulty has been experienced in finding trees characteristically affected in any of the sections devoted to apple growing, it is believed that this rootrot is generally

[1] Published with the permission of the director of the North Carolina Experiment Station.
[2] Fulton, H. R., and Cromwell, R. O. Black rootrot of apple. (Abstract.) *In* Phytopathology, v. 6, no. 1, p. 110. 1916.

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present in the orchards of the western parts of the State and to some extent in other parts.

The same disease, no doubt, occurs in other States. Fulton and Cromwell[1] report its occurrence in Pennsylvania, and Fromme and Thomas[2] have reported it from Virginia.

LOSSES FROM ROOTROT

No approximation has been made of the losses occasioned by this rootrot. Several very obvious reasons can be mentioned to show that the assemblage of such data is next to impossible. In the first place, the symptoms in the above-ground parts of affected trees, as will be made evident later in this report, are not sufficiently characteristic to separate this rootrot from other diseases in which the root system is impaired. In order, therefore, to be certain of a field diagnosis, it is necessary to remove the soil from the collar and roots of suspected trees. Owners of orchards are generally reluctant to have any considerable number of their declining trees disturbed in this manner. In one orchard, however, 10 unhealthy trees were examined in this manner, and 5 were found to be characteristically affected. The disease was found to be generally present on a few trees in the considerable number of other orchards which were examined. It can only be said with reference to losses from this root disease that a small number of trees each year succumb in every orchard in which this trouble is present. These trees range in age from 8 to 30 years.

APPEARANCE OF THE DISEASE

As no part of the causal organism has been found to appear above the surface of the ground, there is no evidence of the presence of rootrot until after the disease has become well established and a considerable number of roots are involved in decay. At this stage the most prominent symptom among the above-ground organs is revealed by the foliage. If it is borne in mind that the destruction of roots proceeds gradually and that affected trees may live for several years, it will be realized that the symptoms change as the disease advances. The leaves on trees whose root systems are in intermediate stages of decay are generally quite normal in size, but are fewer in number than on healthy trees. In more advanced stages the leaves are sometimes as small as one-third the normal size. The progressive inability of diseased trees to store up sufficient reserve food very probably accounts for this production of undersized leaves. In addition, various degrees of chlorosis are always manifest.

Certain abnormalities in fruit production commonly accompany the presence of abnormal foliage. The one most generally noted is the

[1] Fulton, H. R., and Cromwell, R. O. Loc. cit.

[2] Fromme, F. D., and Thomas, H. E. The rootrot disease of the apple in Virginia. *In* Science, n. s., v. 45, no. 1152, p. 93. 1917.

occurrence of an excessively large set of small fruits, a phenomenon frequently displayed by plants weakened by disease.[1] In the following season such trees may produce only a few flowers, and only a score, or even a less number, of fruits are set. The apples on affected trees never develop to normal size, but ripen prematurely. Certain varieties may exhibit a reddish flush when they are no larger, perhaps, than an inch in diameter, and normal apples of the same variety on trees near by will be considerably larger and entirely green.

All of the foliage and fruit throughout affected trees usually exhibit these symptoms equally strikingly. A few cases have been observed, however, where the fruit and leaves on one or two limbs only manifested symptoms of disease; and in such cases the roots are quite normal, except on the side corresponding to the one which bears the abnormal parts.

The effect of this disease upon the twigs is indicated by a decrease in the annual increment of growth when comparison is made with healthy trees of the same variety grown under the same conditions. This decreased growth would naturally be expected to follow any impairment of the root system. In consequence of it there is a more or less marked bunching or rosetting of the leaves.

The roots of affected trees are covered with a thin, compact growth of mycelium, which is snowy white at first; after a few days, however, the superficial portion develops into a black incrustation or stroma. Under conditions favoring the optimum development of the fungus this stroma is sufficiently thick so that the outer black crust may be separated from the snowy-white weft beneath without at the same time removing any of the cortical tissues of the root. Minute, black, threadlike rhizomorphs are seen to radiate from the margin of the stroma and extend for several inches along the surface of the root. These rhizomorphs may anastomose more or less, forming a network, and are in such intimate relation with the cortex that it is impossible to separate intact even small portions. These strands become obscured in the stroma, except in its most recent growth. The disintegration of the cortex beneath the stroma proceeds rapidly and follows closely upon its advance. The bark then becomes fissured, and, when it has dried, it can be readily crumbled. Affected roots are soon girdled, and the distal portions die. New roots are sometimes developed above the diseased parts, thus enabling diseased trees to live for a term of years.

Observations made in the field indicate that soil type, drainage, exposure, elevation, age, and variety of trees seem to have no bearing upon the presence of the disease, since rootrot has been found under the widest variation of these factors. Diseased trees occurred in fields that had been in cultivation for several years before the orchards were set.

[1] Schrenk, Hermann von. A root rot of apple trees caused by Thelephora galactina Fr. *In* Bot. Gaz., v. 34, no. 1, p. 65. 1902.

Trees may remain in normal health for 15 to 30 years before becoming diseased if set soon after the timber has been removed. Trees in orchards which are well cared for appear to be as subject to disease as those in neglected orchards. Not more than four trees in a group have been found to be diseased, and more often such trees stand singly. It is indicated that the dissemination of the disease is accomplished by such agencies as cultivation, rodents, and surface washing of the soil.

EFFECTS OF ROOTROT UPON THE WOODY TISSUES

The effects upon the woody tissues of the roots are much less profound than upon the bark and are macroscopically evident as a uniform brown discoloration. In order to secure a better knowledge of the relation of the fungus to the wood, diseased roots were embedded in celloidin, sec-

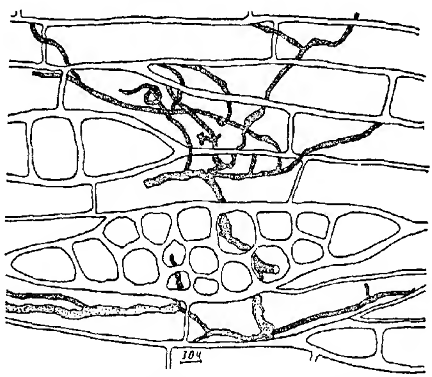

FIG. 1.—Longitudinal section of a diseased apple root. The hyphæ appear to traverse all of the xylem tissues equally well and in all directions.

tioned, and stained. The hyphæ appear to traverse all of the xylem tissues equally well and in all directions, as indicated in figure 1. They may follow the course of the vessel or medullary ray for some distance before diverging into adjacent cells. Perforation of the cell wall appears to be accomplished at any point, and no evidence has been noted that the pores serve as the places of passage. No considerable delignification occurs, as is shown by a comparison of normal and diseased wood when cross sections are treated with phloroglucin and hydrochloric acid. Starch very rapidly disappears, however, from invaded tissues, as is indicated by its absence when sections are tested with iodin. Confirmation of the digestion of starch by this fungus was secured by growth on starch agar. A halo extending beyond the margin of the colony resulted on this medium, thus affording an ocular demonstration of the excretion of amylase. Further evidence of the activity of this organism was

sought by the cultural methods employed by Crabill and Reed [1] in which the carbon-containing compounds cellulose, amygdalin, fibrin, albumin, peptone, casein, and asparagin were added to stock agar of inorganic salts. Although the fungus grows slowly on nutrient agar, well-defined halos had formed within a few days on casein and fibrin agar. Asparagin agar, acidified with hydrochloric acid and made yellow by the addition of rosolic acid, is changed to red by ammonia liberated in the decomposition induced by the organism. The growth on cellulose, amygdalin, and albumin agar indicates that these compounds are not utilized by the fungus. Even though a good growth on peptone resulted, no halo was formed. The results with casein, fibrin, and asparagin indicate that certain proteolytic enzyms (erepsin, protease, and amidase) are secreted.

CAUSE OF THE DISEASE

Isolations have been made at various times from diseased roots from several sources which have constantly yielded an organism with a very characteristic mycelial growth. These cultures remained sterile until the summer of 1915, when conidial fructifications appeared in certain of them. It was not definitely proved, however, that these conidia were those of the causal organism until several months later. Stromatic arms, like those of certain species of Xylaria, formed in these cultures (Pl. 3). The conidia, too, not unlike those of species of this genus, were formed either on these arms or on elevations arising from the incrustation on the surface of the culture medium.[2]

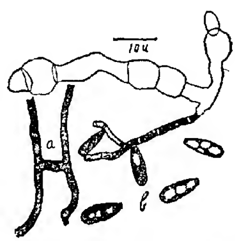

FIG. 2.—*a*, *Xylaria* sp.: Hyphæ, showing the fusion occasionally noted. *b*, The conidia are hyalin, elongated oval in outline, with a blunt truncate pedicel, and measure about 10 by 3 to 3.5 µ. They are borne singly as lateral buds from the sporogenous hypha.

The conidia are hyalin, elongated oval in outline, with a blunt truncate pedicel, and measure about 10 by 3 to 3.5 µ. They are borne singly as lateral buds from the sporogenous hypha (fig. 2, *b*). As yet all attempts to germinate them have been fruitless.

Perithecia have never appeared in any of these cultures, even though a variety of media have been employed under several sets of environmental conditions. Perithecial stromata have been found, however, upon the roots of trees which had succumbed to rootrot. Thus far, all attempts to germinate the ascospores have been unsuccessful, so that it has been impossible to determine by growth in culture or by inoculations

[1] Crabill, C. H., and Reed, H. S. Convenient methods for demonstrating the biochemical activity of microorganisms, with special reference to the production and activity of enzymes. *In* Biochem. Bul., v. 4, no. 13, p. 30–44, pl. 1, 1915.

[2] Specimens from cultures were submitted for examination to Prof. George F. Atkinson, who stated that the organism was probably a species of Xylaria.

whether this perithecial form is genetically connected with the conidial stage which appeared in culture. The perithecial material is morphologically quite like *Xylaria hypoxylon*, a species in which is included a wide variety of forms. It is quite probable that several species of Xylaria are involved in this disease, as has been suggested by Fromme and Thomas,[1] who found *Xylaria polymorpha* associated with apple rootrot in Virginia.

CULTURAL CHARACTERS

Various kinds of nutrient agar were found to be unfavorable substrata, since on them growth proceeded slowly and there was only a slight tendency toward the formation of a black incrustation and rhizomorphs. When agar added to decoctions of apple fruits or roots was employed, and also when sterilized, well-moistened apple roots were used as a culture medium, an abundant mycelium and a well-developed incrustation were produced. No fructifications have been noted on these media, however, even in cultures which have been kept growing for three years.

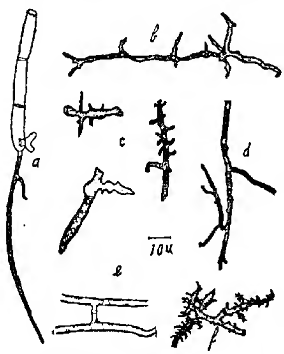

FIG. 3.—*Xylaria* sp.: Mycelium, showing (*a*) considerable variation in the filaments; *b*, *d*, the young filaments are hyalin, branched, granular, and highly refractive, with an average diameter of 2 to 3 μ; *c*, *f*, the hyphæ later lose their granular contents, become brown to olivaceous in color, and numerous blunt spinulose terminals are developed; *e*, fusion of hyphæ.

The general appearance of the mycelium on various culture media is quite like that on decaying apple roots. The young filaments (fig. 3, *b*, *d*) are hyalin, branched, granular, and highly refractive, with an average diameter of only 2 to 3 μ. Considerable variation in the diameter of the filaments occurs, as indicated in figures 2, *b*, and 3, *a*. Later, the hyphæ lose their granular contents, become brown to olivaceous in color, and numerous blunt, spinulose terminals are developed (fig. 3, *c*, *f*). Filaments in old cultures become more closely septate, the cells may attain a diameter of 4 to 8 μ (fig. 2, *b*) and are chlamydospore-like, in that they retain their vitality even after desiccation for several months. Fusion of hyphæ, as shown in figures 2, *a*, and 3, *e*, has occasionally been noted.

On potato plugs, steamed corn meal in flasks, bean pods, and other sterilized plant parts, a copious mycelial growth is produced, which may become crustlike; and, in addition, stromatic arms and conidia are formed. Conidial formation, in reddish brown or grayish sporodochia-

[1] Fromme, F. D., and Thomas, H. E. Loc. cit.

like masses, occurs most profusely on the incrustation on corn meal. Bean pods or corn meal in flasks appear to be most favorable for the formation of stromatic arms. These stromatic arms vary in size from very small to those having a length of 10 to 12 cm. and a diameter of 1 cm. They are either entire or branched in a coralloid manner. The stromata are flesh-colored within and are generally covered, except at the tip, which is also flesh-colored, with a dense hirsute coating. These hairs are 3 to 5 mm. long and impart to the stromata a beautiful variety of colors, among which are gray and shades of brown, violet, and green. Small stromata may not possess this coating of hairs.

A study has been made of the influence of temperature, light and darkness, and moisture upon the growth of this organism. For this purpose, several sets of cultures were prepared, one of which was maintained in an incubator at 37° C. Scarcely any growth takes place at this temperature. A moderate growth of mycelium, conidia, and stromatic arms 4 to 6 cm. long were produced in another set kept at 28°. Room temperatures of 21° to 25° were found to be more favorable for growth than this last temperature, but the most luxuriant growth occurred in cultures kept in an ice box at temperatures of 11° to 13°. The largest stromatic arms obtained were formed at this temperature.

Light appears to exert no morphogenic stimulus in the formation of stromatic arms, since these structures, both simple and branched, appeared in sets of cultures kept in a photographic dark room. The vegetative growth, too, appears to be as luxuriant in darkness as in the light.

In the tests upon the influence of moisture, flask cultures containing 12 gm. each of corn meal were employed. This corn meal was moistened by the addition of quantities of water varying from 20 to 65 c. c. After inoculation the cultures were incubated for six weeks at room temperature. As judged by the luxuriance of growth, 40 to 50 c. c. of water appear to be the optimum quantity. No conidia formed in the cultures containing less than 40 c. c. of water.

PATHOGENICITY

Four apple trees only were used in making tests to determine the parasitism of this organism. On one tree three inoculations were made; on each of two others, six; and on the other, ten. These inoculations were made at West Raleigh, N. C., during the months of May, June, and September. The soil was first removed so as to expose the roots, after which the inoculations were effected. The inoculum, which consisted of mycelia from steamed-rice cultures, was either inserted into wounds made by scraping off the cortex, or by making incisions into it, or was applied to uninjured roots. The places of inoculation were then covered with a layer of soft paraffin. The roots inoculated varied in diameter from $\frac{3}{16}$ to 3 inches. Infections were uniformly successful, irrespective

of whether or not the tissues were injured. The progress of the disease, however, varied considerably. In some cases small areas only had become involved in decay within six weeks, and in others the disease had advanced several inches to a foot or more beyond the point of inoculation. Even though relatively few inoculations have been attempted, the evidence in hand shows beyond doubt that the organism is to be regarded as a vigorous pathogene.

SUMMARY

(1) A little-known apple rootrot which causes the death of trees has been more or less continuously investigated for the past three years.

(2) It has been observed to occur in six widely separated counties in North Carolina and in all probability is the same disease which has been observed in sections of Virginia and Pennsylvania.

(3) The symptoms manifested by the above-ground parts of affected trees do not serve to distinguish this disease from other apple rootrots.

(4) The roots, however, are characteristically covered with black fungus incrustations from whose margins radiate minute, black rhizomorphs. The cortex is quickly corroded, and the roots are girdled while disintegration of the woody portions proceeds slowly.

(5) Isolations have constantly yielded a form whose conidial fructifications and stromatic arms indicate its relationship to *Xylaria* spp.

(6) The ascigerous stage of a species of Xylaria has been found upon diseased apple roots, but has not been proved to be connected with the conidial stage developed in artificial culture.

(7) Mature mycelium in culture possesses numerous characteristic spinulose branches.

(8) The optimum development of the organism seems to be obtained when temperatures of 11° to 13° C. are maintained either in the presence or absence of light.

(9) Pathogenicity is established by inoculation with pure cultures into the roots of living apple trees.

PLATE 3

A.—Stromatic arms formed in cultures on steamed corn meal; resembling those of certain species of Xylaria. They may form in abundance on this medium. Excreted drops give the arms a scarred appearance. About natural size.

B.—A few stromatic arms from the same culture. About natural size.


A
B

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